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Cross-sex pattern of bone mineral density in early onset gender identity disorder

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Abstract

Hormonally controlled differences in bone mineral density (BMD) between males and females are well studied. The effects of cross-sex hormones on bone metabolism in patients with early onset gender identity disorder (EO-GID), however, are unclear. We examined BMD, total body fat (TBF) and total lean body mass (TLBM) in patients prior to initiation of sex hormone treatment and during treatment at months 3 and 12. The study included 33 EO-GID patients who were approved for sex reassignment and a control group of 122 healthy Norwegians (males, $n=77$; females, $n=45$). Male patients ($n=12$) received an oral dose of 50 μg ethinylestradiol daily for the first 3 months and 100 μg daily thereafter. Female patients ($n=21$) received 250 mg testosterone enantate intramuscularly every third week. BMD, TBF and TLBM were estimated using dual energy X-ray absorptiometry (DXA). In male patients, the DXA measurements except TBF were significantly lower compared to their same-sex control group at baseline and did not change during treatment. In female patients, the DXA measurements were slightly higher than in same-sex controls at baseline and also remained unchanged during treatment. In conclusion, this study reports that body composition and bone density of EO-GID patients show less pronounced sex differences compared to controls and that bone density was unaffected by cross-sex hormone treatment.

Keywords: Gender identity disorder; Bone metabolism; BMD; Estrogen receptors; Estrogen; Testosterone; Gonadotropin

Introduction

Sex is the major predictor of and contributing factor to skeletal size and shape. Evidence from twin and family studies suggests that between 50 and 85% of the variance in peak bone mass (bony tissue present at the end of the skeletal maturation) is genetically determined (Gueguen et al., 1995; Krall and Dawson-Hughes, 1993). Many candidate genes have been identified that regulate bone mass and susceptibility to osteoporosis, but the full profile of such genes and their variants remain to be defined (Ralston and de Crombrugghe, 2006). Furthermore, bone mineral density (BMD) has also been found to be determined by environmental factors such as diet and lifestyle (Falch, 1982; Lewiecki, 2005). Nevertheless, gonadal hormones have an important modulating impact on bone physiology in both sexes (Turner et al., 1995). Prior to puberty, boys and girls gain BMD at similar rates. After puberty, men tend to acquire greater BMD than women (overview provided by the National Institute of Arthritis Musculoskeletal and Skin Disease at The National Institute of Health, www.niams.nih.gov).

The propose role of sex hormones, such as estrogens, in bone physiology is supported by the marked bone loss seen at menopause or after castration, a process mediated by increased osteoclastic bone resorption (Riggs et al., 1998). Menopause leads to accelerated bone loss that plateaus after 5 to 10 years (Gennari et al., 2002). Conversely, several studies have shown a significant effect of estrogen substitution on reduction of bone loss and fracture risk in postmenopausal women (Lindsay,

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2004). Moreover, estrogens have a strong influence on both fetal bone growth and on postnatally imprinted bone cells (i.e., pubertal growth or closure of the epiphyses) (Couse and Korach, 1999; Manolagas et al., 2002; Parfitt, 2002).

The role for androgens in bone physiology is supported by reduced BMD seen in androgen-deprived prostate cancer patients (Basaria et al., 2002; Wakley et al., 1991). Withdrawal of testosterone has been shown to increase osteoclast activity and thus increase bone loss (Bellido et al., 1995). Conversely, testosterone substitution increases BMD in cases of male hypogonadism (Katznelson et al., 1996), possibly by stimulating osteoblast proliferation and differentiation, thereby increasing matrix production and mineralization (Kasperk et al., 1989, 1997; Vanderschueren and Bouillon, 1995).

Estrogens and androgens appear to act synergistically on bone metabolism in both sexes (Kasperk et al., 1989; Zumoff et al., 1995). Patients with complete androgen insensitivity experienced pubertal growth when treated with estrogens, suggesting that estrogens are sufficient to induce pubertal growth in these patients when prematurely castrated (Zachmann et al., 1986). Furthermore, anabolic steroid treatment increases bone density in postmenopausal women (Kanis et al., 1992). Finally, hyperandrogenism appears to limit bone loss in female patients with polycystic ovary syndrome (Adami et al., 1998).

In both sexes, estrogens act through estrogen receptors alpha and beta (ER and ER). Males homozygous for an inactivating mutation of the *ER* gene show increased osteoporosis (Smith et al., 1994). A similar effect is described in men with an inactivating mutation of the gene encoding aromatase, the enzyme that converts androgens to estrogens (Bilezikian et al., 1998; Carani et al., 1997; Morishima et al., 1995). It is therefore likely that gene polymorphisms in hormone receptors and relevant enzymes play an important role for bone density in normal males and females (Gennari et al., 2002; Niu and Rosen, 2005; Valero et al., 2005). Estrogens are known to act directly with their receptors on osteoblasts, bone marrow stromal cells and osteoclasts (Bland, 2000; Cutler, 1997; Fiorelli et al., 1995; Grumbach and Auchus, 1999). One effect of estrogens is an increase in the absolute number of osteoblasts present in the bone marrow (Majeska et al., 1994), another is an indirect effects on osteoclasts by suppressing the production of bone-resorbing cytokines from osteoblasts and bone marrow stromal cells (Pacifici, 1998).

Physical activity is an important factor in modulating bone density (Frost, 1987, 1990; Schiessl et al., 1998). It is difficult, however, to separate the direct effects of exercise on BMD from the indirect effects of exercise-associated improvements in mobility and balance, which simply decreases the risk of falling (Fiatarone et al., 1990; Gass and Dawson-Hughes, 2006). High-impact physical training correlates with increased BMD, but low-impact exercise, such as walking, neither increases BMD nor reduces fracture risk (Going et al., 2003). It is postulated that exercise during pre-pubertal years and adolescence builds a skeleton with a high bone mineral density (BMD) and possibly a larger skeleton with different skeletal architecture (Nordstrom et al., 2005; Seeman, 2005). However, unlike the BMD calculated by DXA (g/cm^2), the true volumetric bone mineral density (g/cm^3) does not appear to increase with size and/or age. Variations in bone parameters during childhood and adolescence reflect changing growth and increased size rather than increased bone mineral per unit volume.

Increased remodeling rate is physiologic for rapid growth during puberty but could be pathologic in the mature skeleton (Manolagas et al., 2002). Unfortunately, no randomized, prospective study has documented an activity-associated reduction of fracture frequency to date (Karlsson, 2004). Nevertheless, BMD loss as a result of changing gravity in long-duration spaceflight strongly implies that mechanical input is indeed required for its maintenance density (Iwamoto et al., 2005; Lang et al., 2006).

Lean body mass and adipose tissue distribution also differ between men and women (Ross, 1996; Ross et al., 1994; Vogel and Friedl, 1992), and cross-sex estrogen and testosterone treatment induce changes in the typical gender-specific fat and muscle distribution patterns (Elbers et al., 1997, 1999). Cross-sex hormone treatment, as a current mode of intervention in adult gender identity disorder (GID) patients, results in considerable somatic changes. Several authors have reported that BMD and lean/adipose tissue distribution might be altered in a "cross-sex manner" (in parallel with changes in sex hormone levels; males showing female and females male values) as a result of such treatment (Dittrich et al., 2005; Goh and Ratnam, 1997; Lips et al., 1996a,b; Reutrakul et al., 1998; Ruetsche et al., 2005; van Kesteren et al., 1998).

In this study, we investigated potential variations in BMD and lean/adipose tissue distribution in a representative group of untreated Norwegian early onset GID patients compared to a Norwegian young adult reference group. Furthermore, we studied the longitudinal effects of cross-sex hormone treatment on these parameters in this particular patient group.

Subjects and methods

Inclusion criteria

Thirty-three somatically healthy EO-GID patients with symptom onset at <12 years of age and who consecutively sought sex reassignment surgery (SRS) in Norway between 1996 and 1999 were included (21 females and 12 males). Patients' age ranged from 20 to 46 years of age (21 females, mean age (SD)=25.1 (4.8) years and 12 males, mean age (SD)=29.3 (7.8) years). All patients were evaluated according to the Harry Benjamin International Gender Dysphoria Association's Standards of Care [1990 and 1998 (Levine et al., 1998)]. In two independent comprehensive evaluations through structured interviews with two individual senior psychiatrists, all patients fulfilled criteria A to D in DSM-IV from childhood on (<12 years of age). From the perspective of their sex, their sexual preferences at the time of investigation were distributed as follows: (1) attracted to the same-sex only (n=30), (2) attracted to both sexes (n=2) or (3) attracted to neither sex (n=1).

Controls

A group of 122 young persons in the same age range consisting of 77 males (age (SD)=33.9 (9.3)) and 45 females (mean age (SD)=38.6 (6.1)) were selected as controls from a Norwegian reference population for National BMD, total body fat and lean body mass determination (n=372) (Falch, 2004; Falch and Meyer, 1996; Faulkner et al., 1996).

Exclusion criteria

All participants were free of any medication and alcohol or drug abuse. Applicants with any endocrinological, genetic, neurological or major psychiatric co-morbidity were excluded at baseline (two delusional disorders, one XXY anomaly).

All participants signed an informed consent form.

Techniques

Dual-energy X-ray absorptiometry (DXA) is the most widely used technique for estimating bone mass (Genant et al., 1996) and body composition (TBF and TLBM Van Loan, 1998). Utilizing differing energy levels of the X-ray beams, the absorption of the radiation is determined. Bone mineral density (BMD, g/cm^2) was measured in the spine (L2–4), left femoral neck and total body using Lunar DPX-l (Lunar, Wisconsin, USA). The coefficient of variation was calculated for L2–4 (1.0%), femoral neck (2.5%), and total body (0.7%). Bone mineral content (BMC) was determined as bone mass (g) without correction for bone size. TBF and TLBM were determined from all body fat and lean body measurements.

Design

The bone density and biochemical variables of bone metabolism of GID patients were determined at baseline, 3 months, and 12 months after the initiation of cross-sex hormone treatment.

Laboratory methods

Blood samples were drawn between 9 AM and 12 noon at baseline, 3 months and 12 months after initiation of cross-sex hormone treatment. At baseline, the blood samples from female participants were drawn without regard to the phase of menstrual cycle. The 3- and 12-month samples were obtained 1 week after the most recent testosterone injection. All serum samples were stored at −20 °C until measurement at the Hormone Laboratory, Centre of Endocrinology, Aker University Hospital, Oslo. Serum concentrations of estradiol (E_2), progesterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), free thyroxine (FT_4), thyroid-stimulating hormone (TSH), and prolactin (PRL) were determined by time-resolved fluoroimmunoassays (Delfia, Wallac, Turku, Finland). Serum concentrations of cortisol and sex hormone binding globulin (SHBG) were measured by luminoimmunoassays (DPC, Los Angeles, USA) and testosterone was measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland). Dihydrotestosterone (DHT) and estrone (E_1) were measured by in house radioimmunoassays. The normal ranges for adults established in our laboratory were as follows: E_1, males 0.10–0.28, females 0.11–0.45 nmol/l; E_2, males 0.08–0.14, females 0.08–0.85 nmol/l; FSH and LH, <12 IU/l; free T4, 8–20 pmol/l; TSH, 0.5–3.6 IU/l; PRL, 50–700 mIU/l; cortisol (08.00), 250–750 nmol/l; progesterone, males <3, females in follicular phase <3, in luteal phase >15 nmol/l; SHBG, males 10–60, females 30–90 nmol/l; testosterone, males 8–35, females <3 nmol/l; and DHT, males 0.9–3.1, females 0.2–1.00 nmol/l. Since total testosterone measurements do not adequately reflect the free concentration of testosterone in the presence of altered SHBG levels, a free testosterone index (FTI) was calculated (FTI = testosterone × 10/SHGB). The normal ranges for FTI were 0.1–0.6 for females and 2.3–9.9 for males.

The serum levels of osteocalcin were measured by luminoimmunoassay (B.R.A.H.M.S. Diagnostica GMBH, Berlin, Germany), C-terminal telopeptide of type 1 collagen generated by metalloproteinases (1CTP) by radioimmunoassay (Orion Diagnostica, Espoo, Finland) and bone-specific alkaline phosphatase (bALP) by enzyme activity measurement (Metra Biosystems Inc., CA, USA). The urine concentrations of deoxypyridinoline cross-links (D- PYR) were measured by enzymeimmunoassay (Metra Biosystems Inc., CA, USA) and N- telopeptides type I collagen (NTx) by enzymeimmunoassay (Ostex, Seattle, WA, USA).

The normal serum ranges for adults were as follows: osteocalcin, males 0.7–2.6, females 0.6–3.4 nmol/l; bALP, males 15–41, females 12–31 U/l; and 1CTP, males 2.1–5.6, females 2.1–5.0 μg/l. The normal urine ranges for adults were as follows: D-PYR, males (18–55 years) 2.3–5.4, females (18–45 years) 3.0–7.4 nmol/l D-PYR/mmol/l creatinine; and NTx, males (25–49 years) <65, females (25–49 years) <65 nmol/l BCE/mmol/l creatinine.

Treatment

All male EO-GID patients undergoing treatment (M to F) received 50 μg of oral ethinylestradiol (Etifollin) daily during the first 3 months of treatment and thereafter 100 μg daily. All female EO-GID patients undergoing treatment (F to M) received 250 mg testosterone enantate (Primoteston-Depot) intramuscularly every third week.

Table 1
Hormones and biochemical characteristics of the study subjects and their changes over time

	GID patients							
	Females (n=21)				Males (n=12)			
	Baseline	3 months	12 months	p-values *	Baseline	3 months	12 months	p-values
E1 (nmol/l)	0.4 (0.2)	0.4 (0.2)	0.3 (0.1)	0.6	0.3 (0.2)	0.6 (0.7)	0.6 (0.7)	0.16
E2 (nmol/l)	0.4 (0.2)	0.2 (0.2)	0.2 (0.1)	<0.0005 *	0.1 (0.1)	0.2 (0.2)	0.2 (0.2)	0.67
FSH (IU/l)	8.0 (12.3)	7.1 (12.7)	4.7 (4.1)	0.09	4.7 (3.4)	2.2 (3.1)	1.7 (1.6)	0.02 *
LH (IU/l)	14.8 (15.5)	7.8 (11.1)	6.0 (6.3)	0.02 *	6.0 (3.3)	2.2 (1.8)	2.4 (2.7)	0.04 *
Progesterone (nmol/l)	10.8 (15.5)	2.3 (5.1)	1.4 (1.4)	0.006 *	1.5 (0.6)	2.0 (3.0)	2.4 (3.6)	0.29
SHGB (nmol/l)	48.1 (21.6)	27.6 (15.6)	26.3 (16.5)	<0.0005 *	32.4 (17.3)	151.9 (96.8)	189.8 (100.2)	<0.0005 *
DHT (nmol/l)	0.6 (0.4)	1.2 (0.8)	1.8 (0.7)	<0.0005 *	1.3 (0.6)	0.8 (1.0)	0.6 (0.7)	0.31
Testosterone (nmol/l)	3.2 (7.6)	23.3 (11.8)	29.2 (12.0)	<0.0005 *	16.8 (9.7)	9.0 (14.9)	6.8 (9.0)	0.02 *
FTI (Tx10/SHBG)	0.7 (0.4)	8.4 (4.4)	11.1 (3.8)	<0.0005 *	5.2 (2.0)	0.6 (0.4)	0.4 (0.2)	0.006 *
Prolactin (mIU/l)	219.0 (84.1)	220.5 (133.6)	194.2 (80.8)	0.03 *	174.3 (87.8)	213.2 (96.5)	212.3 (87.5)	0.23
Cortisol (nmol/l)	348.4 (161.0)	288.0 (172.2)	301.2 (114.5)	0.21	473.9 (145.1)	648.0 (286.3)	629.3 (249.7)	0.12
Osteocalcin (nmol/l)	2.2 (0.6)	2.0 (1.2)	1.9 (0.4)	0.26	2.7 (0.8)	1.2 (0.9)	0.7 (0.3)	<0.0005 *
BALP (U/l)	15.78 (4.5)	20.1 (6.4)	20.7 (6.9)	<0.005 *	43.3 (26.7)	29.3 (14.0)	14.3 (1.8)	0.01 *
1 CTP (μg/l)	5.1 (2.4)	6.4 (1.9)	6.7 (1.3)	0.001 *	4.5 (0.9)	4.4 (1.3)	2.5 (0.8)	0.61
D-PYR nmol/nMCr	8.0 (2.01)	7.7 (1.8)	6.4 (1.2)	0.49	5.2 (1.76)	6.2 (1.8)	4.7 (1.1)	0.006 *
NTx (nmol/lBCE/mmol/lCr)	68.6 (44.01)	62.2 (29.5)	54.2 (29.2)	0.26	115.3 (61.6)	72.7 (37.4)	40.3 (22.6)	0.03 *

Data are presented as means (SD).
Abbreviations are explained in the Subjects and methods section.
* p-values (significance level<0.05) based on paired sample t-test baseline vs. 12-month values.

Statistical analysis

All statistical analyses were done with the Statistical Package of Social Science program, version 14 (SPSS, 2006).

In the first step of baseline DXA analyses, the control (C) group and GID patients were compared by sex. In addition, we used Cohen's *d* to evaluate effect sizes in this two sample context. Advice on the interpretation of Cohen's d differs, and we follow the original suggestion in Cohen (1992): where 0.2 is indicative of a small effect, 0.5 of a medium and 0.8 of a large effect size. In the second step, non-parametric tests and unadjusted one-way ANOVAs (Altman, 1991; SPSS) (or, equivalently, two-sampled *t*-tests when only two groups were involved) were applied to compare GID patients with controls. ANOVA was accomplished using the univariate option of SPSS. When continuous covariates are entered into the model, this corresponds to analysis of covariance. The BMD, TBF and TLBM measurements served as dependent variables. The predictors were group (C, GID); sex and age (years) were analyzed and adjusted for each other. Tests for homogeneity were performed. All interactions were tested, and the interaction between group and sex was listed in the tables because of its importance for this study.

In the third and final step of analysis, the changes in dependent variables over time were analyzed only in the early onset GID group using summary statistics (Matthews et al., 1990). *Delta03* (calculated by dividing the difference between values at 3 months and baseline by 3) and, similarly, *delta12* (based on baseline and 12 months' values) were used as summary statistics. The results did not differ and the assumption of linear change was accepted. The *delta12* variables were used in further one-way ANOVAs as dependent variables, and the potential predictors' influences on the test result were analyzed. Throughout the report, *delta* indicates a change calculated from baseline and 3 or 12 months' values. For instance, *deltaLH* denotes the difference between LH values after 12 months and baseline divided by 12. Dividing is done to enable comparison of 3 and 12 months' changes, having no effect on the assessment of *p*-values.

In addition to the analyses discussed above, Pearson and Spearman correlations were calculated between the changes of the hormonal variables and the BMD, TBF and TLBM variables over time.

Results

The endocrinological data

The biochemical data of GID patients are summarized in Table 1. Prior to treatment, both M to F and F to M GID patients shared characteristics similar to their specific biological sex group. Female patients showed normal LH and progesterone values related to their menstrual cycles (see also Subjects and methods).

Ethinylestradiol treatment led to a significant decrease in testosterone levels and a 5-fold increase in SHBG levels in M to F GID patients, causing a reduction of FTI from normal male (5.2) to normal female values (0.6) within 3 months. Similarly, there was an expressed decrease in the total concentration of DHT, and therefore the reduction of free DHT concentration was pronounced. Only small increases were found in serum levels of E_1 and E_2, as the assays used do not detect the exogenous ethinylestradiol. However, the biological effects of the estrogen treatment were clearly demonstrated by highly significant increases in serum SHBG levels in the M to F GID patients. Accordingly, the *p*-values in paired sample *t*-test for testosterone, FTI and SHGB were highly significant (Table 1, column 8).

In the F to M early onset GID patients, testosterone treatment led to a significant increase in testosterone concentrations from normal female to normal male levels within 3 months and a

Table 2a
Baseline BMD, total body fat and lean body mass data in 33 early onset GID patients and 122 controls

	BMD L2–4 (g/cm^2)		BMD femur neck (g/cm^2)		Total body BMD (g/cm^2)		Total body BMC (g)		Total body fat (g)		Total lean body mass (g)	
	C	GID	C	GID	C	GID	C	GID	C	GID	C	GID
Male												
Means	1.26 (0.15)+	1.13 (0.16)	1.10 (0.15)	1.04 (0.17)	1.25 (0.09)	1.18 (0.12)	3413 (457)	3077 (463)	16140 (5664)	26180 (12575)	59059 (5745)	54449 (6184)
t-test C vs. GID	0.01 *, 2.6, 86		0.18, 1.3, 84		0.03 *, 2.3, 83		0.03 *, 2.4, 83		<0.0005 *, −4.5, 83		0.02 *, 2.5, 83	
(*p*, *t*, *df*)												
Female												
Means	1.23 (0.13)	1.24 (0.16)	0.95 (0.11)	1.02 (0.11)	1.17 (0.08)	1.21 (0.08)	2682 (387)	2795 (410)	21331 (7864)	22406 (9020)	41279 (3953)	40818 (4419)
t-test C vs. GID	0.95, −0.06, 62		0.02 *, 2.4, 62		0.10, −1.7, 62		0.30, −1.1, 62		0.63, −0.5, 62		0.68, 0.41, 62	
(*p*, *t*, *df*)												

Means and independent sample *t*-test *p* values for each sex group in GID patients compared to controls.
Data are represented as means (SD = standard deviation).
* *p*-values (significance level <0.05) based on independent sample t-test baseline C vs. GID.

Table 2b
Cohen's *d* as effect size measurement for baseline BMD, total body fat and total lean body mass data in 33 early onset GID patients compared to 122 controls

	BMD L2–4	BMD femur neck	Total body BMD	Total body BMC	Total body fat	Total lean body mass
Male C vs. female C	0.21	1.14	0.94	1.73	−0.76	3.60
Male C vs. male GID	0.84	0.37	0.66	0.73	−1.03	0.77
Female C vs. female GID	−0.07	−0.64	−0.50	−0.28	−0.13	0.11
Male GID vs. female C	−0.70	0.63	0.09	0.93	0.93	2.54
Male GID vs. female GID	−0.70	0.14	−0.29	0.65	0.34	2.54
Male C vs. female GID	0.13	0.61	0.47	1.42	−0.83	3.56

Cohen's *d* definition see Subjects and methods.

simultaneous reduction in SHBG levels. FTI increased 16-fold from normal female (0.6) to normal male values (11.1), and total DHT showed a 3-fold increase. Cortisol binding globulin (CBG) also increased during estrogen treatment and decreased during testosterone treatment, as indicated in Table 1 (showing the changes in cortisol levels). The *p*-values for SHGB, DHT, testosterone, and FTI are similarly very small. Effects on progesterone levels are likely due to LH/FSH and cortisol changes as explained above (Table 1).

Biochemical markers of bone metabolism

In M to F GID patients, bone formation measurements (osteocalcin and bALP) and bone resorption measurements (1CTP and NTx) were 50% lower than in their sex control group at 12 months. The values, however, were within the reference ranges, except for NTx at baseline (Table 1, see also Subjects and methods). In F to M GID patients, significant elevations in bone formation measurements were observed over time.

Height and weight

In M to F GID patients the height and weight differed only slightly from the male control group (mean height (SD), male GID = 179.0 (5.7) cm vs. male controls = 178.9 (6.5) cm; *t*-test: $p = 0.95$, CI −3.9 to 3.7; mean weight (SD), male GID = 81.0 (18.8) kg vs. male controls = 79.1 (10.5) kg; *t*-test: $p = 0.58$, CI −8.7 to 4.7). Moreover, only small differences between female GID patients and their sex control group were observed (mean height (SD), female GID = 167.2 (6.6) cm vs. female controls = 165.1 cm (6.5 cm); *t*-test: $p = 0.2$, CI −5.2 to 1.0; mean weight (SD), female GID = 66.4 (11.7) kg vs. female controls = 66.6.(11.3) kg; *t*-test: $p = 0.9$, CI −5.1 to 5.7).

Cross-sex differences of BMD in GID patients

The BMD, TBF and TLBM data were compared between the GID patients prior to hormone therapy and their respective comparison groups of the same sex (Tables 2a and 2b). Despite normal baseline hormonal status of the M to F GID patients, their BMD status of lumbar spine, total body BMD, total BMC, TBF and TLBM was different. The values were significantly lower, apart from TBF which was significantly higher in the GID patients compared to 77 male controls (Table 2a).

F to M GID patients' status was similar to the controls, except for a significantly higher femoral neck BMD (Table 2a).

The influence of sex and group on the results

Table 2b shows the Cohen's effect sizes calculated from the values of Table 2a, comparing different sex groups of controls and patients, and several conclusions and observations were apparent from the data. First, normal differences were observed between males and females of the control group with higher values for males than females in all parameters except total body fat (Table 2b, row 1). Second, male GID patients differed in all parameters, showing lower values for L2–4 BMD, femur neck BMD, total BMD, total BMC, and higher values for total

Table 3
Adjusted ANOVA

Predictor adjusted for the others	BMD L2–4	BMD femur neck	Total body BMD	Total body BMC	Total body fat	Total lean body mass
Sex+ (*p*, *F* (*df* values given if significant))	0.17, 1.86	0.003*, 9.32 (1, 145)	0.09, 2.84	0.0005*, 30.45, (1, 144)	0.68, 0.17	0.0005*, 206.86, (1, 145)
Group+	0.04*, 4.53, (1, 147)	0.08, 3.10	0.19, 1.71	0.20, 2.10	<0.0005*, 20.27, (1, 144)	0.04*, 4.31, (1, 145)
Age++	0.46, 0.54	<0.0005*, 20.28, (1, 145)	0.16, 2.02	0.43, 0.63	0.004*, 8.80, (1, 144)	0.92, 0.01
Group by sex+++	0.06, 3.50	0.14, 2.23	0.02*, 6.03, (1, 144)	0.03*, 5.00, (1, 144)	0.03*, 4.70, (1, 144)	0.06, 3.53

Baseline BMD, total body fat and total lean body mass data in 33 early onset GID patients and 122 controls.
Adjusted ANOVA *p*-values at baseline for each predictor's influence (sex, group, age and the important interaction group by sex, adjusted for each other).
For example, "Total body BMC" differs significantly between the sexes ($p = 0.0005$), but this sex effect also differs significantly between the groups (group by sex $p = 0.03$).
For baseline means, see Table 2a.
Exact *p*-values (statistically significance level $p = 0.05$), *F* and *df* (given when significant) values based on adjusted ANOVA.
Predictors:
+Sex, and Group (C, GID-N) used as contrasted factors.
++Age continually distributed, used as covariate.
+++Group by sex interaction used as well as a predictor.

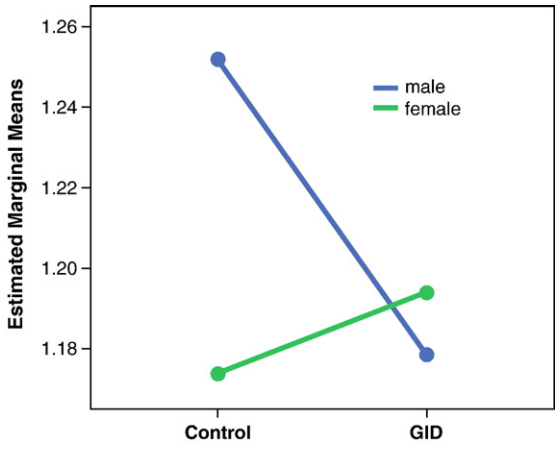

Fig. 1. Estimated marginal means of total BMD (g/cm^2) by group and sex.

body fat compared to male controls (Table 2b, row 2). Third, medium effect sizes for L2–4 BMD and total BMD were found, with higher values for female GID patients compared to female controls (Table 2b, row 3). Fourth, sex differences were less pronounced between male GID patients and female controls and inverse medium/large effects for L2–4 BMD and TBF were observed (Table 2b, rows 4 and 1). Fifth, sex differences between the male and female GID patients were less pronounced than between in the control groups, and an inverse medium effect with higher values for female compared to male GID patients was shown for L2–4 BMD and total body BMD (Table 2b, rows 5 and 1). Sixth, female GID patients compared to male controls showed less sex difference as a result of more masculine values for the female GID patients (Table 2b, rows 6 and 1).

Using adjusted ANOVA, group by sex interactions were significant for total BMD, BMC and TBF at baseline (Table 3), which supported the effect size differences presented in Table 2b. The significant sex by group interactions for several variables at baseline underscored the observation that sex differences in GID patients were less pronounced and the pattern partly reversed (Tables 2b and 3, Fig. 1). Furthermore, we found the well-documented significant correlations between female sex and lower BMD, higher age and lower BMD, as well as higher age and more body fat (Table 3). Different statistical approaches (ANOVA and non-parametric statistics (Mann–Whitney)) generated essentially the same results.

BMD changes over time in GID patients

In the final step of analysis, we investigated the changes in characteristics over time in GID patients. The changes of TBF and TLBM were significantly different for males and females during cross-sex hormone treatment (Table 4). Male GID patients gained significant TBF and lost lean body mass, while females gained significant lean body mass. The BMD parameters remained unchanged from baseline (Table 4). Furthermore and as expected, the TLBM changes correlated with weight changes (Table 4).

Our findings demonstrate a "cross-sex pattern" (EO-GID males show values more similar to female control and vice versa)

Table 4
Changes of BMD, total body fat and lean body mass data in 33 early onset GID patients treated with cross-sex hormones

Mean changes by months and sex+++

	BMD L2–4 Delta(g/cm^2)/*t*		BMD femur neck Delta(g/cm^2)/*t*		Total body BMD Delta(g/cm^2)/*t*		Total body BMC Delta(g)/*t*		Total body fat Delta(g)/*t*		Total lean body mass Delta(g)/*t*	
Months of treatment	3	12	3	12	3	12	3	12	3	12	3	12
Mean changes males	0.02	0.01	0.014	−0.002	0.08	0.009	1.4	0.2	17.3*	5.7*	−8.4*	−3.0*
Mean changes females	0.01	0.001	−0.01	0.0002	0.03	0.01	0.3	0.1	−2.7*	0.6	1.01*	0.37*

Adjusted ANOVA of the mean changes

p-Values controlled for the predictor biological sex and weight of the parameter changes after 3 and 12 months of treatment

	BMD L2–4		BMD femur neck		Total body BMD		Total body BMC		Total body fat		Total lean body mass	
Months	3	12	3	12	3	12	3	12	3	12	3	12
Sex, *p*, *F*, *df* (1, 2)												
P	0.18, 1.88	0.27, 1.3	1.0, 0.009	0.40, 0.74	0.78, 0.08	0.47, 0.57	0.65, 0.21	0.68, 0.26	0.01*, 7.4*	0.01*, 15.51	<0.0005*, 26.34	0.001*, 10.1*
F									(1, 24)	(1, 20)	(1, 24)	(1, 20)
Weight, *p*, *F*, *df* (1, 2)												
	0.21, 1.7	0.44, 0.63	0.40, 0.80	0.40, 0.73	0.54, 0.39	0.96, 0.002	0.16, 2.1	0.62, 0.17	0.25, 1.40	0.22, 1.66	<0.0005, 17.43 (1, 24)	0.08, 3.52

+++Mean change: the Delta value, or slope, corresponding to 12 months, is calculated as explained in the section on statistics by dividing the difference between the values at 12 months and baseline by 12. A similar comment applies to the columns corresponding to 3 months.
p-values (statistically significance level $p=0.05$) *F* and *df* values based on adjusted ANOVA.
Predictors:
+Sex used as contrasted factors.
++Weight continually distributed, used as covariate.

in GID male patients even prior to treatment and suggest a similar trend in females (for example, total body BMD in Fig. 1), although the results in males are more pronounced than for females (Tables 2a and b, 3, Fig. 1). The reduced TLBM and particularly the gained TBF in male GID patients compared to male controls became pronounced during 12 months of hormone treatment.

Discussion

Cross-over sex pattern at baseline

Interestingly, we observed that, even at baseline, male GID patients exhibited more of a female pattern, while female GID patients had a more male pattern in all parameters. Sex differences among GID patients were actually less pronounced than sex differences in the control groups.

In M to F GID patients, we found several BMD measurement, effect size and group by sex interaction differences compared to male controls, as well as atypical sex differences compared to female controls and female GID patients. In F to M GID patients, we found different BMD femur neck measurements, effect sizes and significantly different "group by sex interactions" compared to female controls at baseline. Female patients showed smaller sex differences compared to male GID patients and male controls.

These findings were consistent and independent of the statistical method utilized. Moreover, this "cross-over" pattern remained substantial even after correcting for age differences. The cross-over pattern was most strongly expressed as a significant group by sex interaction in ANOVA or in Cohen's effect size values. These results could not be caused by any known hormonal disturbances, as GID patients had hormonal values similar to their biological sex reference group at baseline.

Treatment effect

Another observation was, following estrogen treatment, the differences in total body fat and lean body mass increased between male GID patients and male controls over time.

By contrast, previous studies (Dittrich et al., 2005; Ruetsche et al., 2005) have demonstrated either an increase of BMD in M to F GID patients during the first year of treatment (van Kesteren et al., 1996, 1998), an initial decrease followed by a later increase (Reutrakul et al., 1998), a decline back to baseline (van Kesteren et al., 1998) or no BMD change (Schlatterer et al., 1998b).

Contrary to the findings in the male GID patients, a significant further gain in TLBM and a loss in TBF were found in the female patients during treatment. These results are partly in agreement with previous studies (Goh and Ratnam, 1997; Schlatterer et al., 1998b; van Kesteren et al., 1998) and support the assumption that testosterone treatment may protect bone from the deleterious effects of estrogen deficiency in these patients (Hierl et al., 1999; Lips et al., 1996a,b).

In addition, the biochemical changes of bone formation and resorption markers showed a clear reduction in male and resorption markers in female patients over time. Although the absolute values of bone resorption were only slightly reduced it might indicate an insufficient bone restoration over time (McClung et al., 2004).

Relationship between endocrine changes and BMD

We found no significant correlations between the changes in gonadotropins (*deltaLH* and *deltaFSH*) and the changes in BMD, although there was a significant difference between the sexes regarding suppression of LH ($p=0.03$). F to M GID patients had higher average LH values than M to F GID patients (Table 1), due likely to obtaining baseline blood samples at different phases during the menstrual cycle in the females, thus causing a larger range of LH than in males.

The importance of the inclusion criteria

Our observations may be an expression of different genetic and environmental factors or their interaction of these factors and might be related to the fact that we strictly only included early onset GID patients. In most studies, diagnostic inclusion criteria are not precisely defined (Dittrich et al., 2005; Michel et al., 2001; Reutrakul et al., 1998, 2005; Schiessl et al., 1998; Schlatterer et al., 1998a).

The previously described higher lumbar spine BMD in F to M compared to M to F GID patients (van Kesteren et al., 1998) was verified in our study. However, no absolute lumbar spine BMD differences between F to M GID patients and female controls were apparent in our study. Nevertheless, we found differences almost in all effect sizes when comparing female patients to female controls, indicating a more masculine pattern in patients at baseline.

Our study does not confirm similarities in serum levels of bone markers of male GID patients with those of the female patients prior to treatment (van Kesteren et al., 1998). Effect size bone formation measurements showed an increase in biological female and decrease in biological male patients, and opposite effects were observed for bone resorption.

Potential explanations of the results

Our results support the model by which receptor polymorphisms might play a role in the genetic regulation of bone mass (Brown et al., 2001; Riancho et al., 2005; Zarrabeitia et al., 2004a,b) and that allelic variants may further increase the risk for osteoporosis (Gennari et al., 2004). In this sense, the pharmacological effects that cross-sex hormone treatment might have on genetic-born males and females are likely to be important. Estrogens exert their effects through alpha and beta estrogen receptors, while androgens exert their effects through both androgen and estrogen receptors (the latter due to conversion of androgen to estrogen by aromatase). Thus, estrogen substitution usually helps to regain the significant loss of BMD in young males suffering from aromatase deficiency (Bilezikian et al., 1998; Smith et al., 1994) (also reviewed in Khosla, 2002). We could not observe a similar effect in our patient group.

One potential confounder in our results could be the distribution of fat. Our male patient group showed significantly higher TBF values than the male controls, and higher total body fat predicts bone mass negatively (Hsu et al., 2006).

Differences in life style of male controls and male GID patients (i.e., less physical activity and higher total body fat) may contribute to the negative bone physiology discussed above. Some studies suggest that male EO-GID patients avoid competitive physical sports, whereas the opposite is suggested for female patients (Bailey and Zucker, 1995; Zucker and Bradley, 1995).

Furthermore, some studies suggest that there might be an intrauterine programming of BMD by the hypothalamic–pituitary axis which would predict adult BMD. Nevertheless, until now there is a relative lack of studies giving evidence for predictive prenatal factors and the EO-GID phenomenon during adolescence and adulthood (Cooper et al., 1995; Dennison et al., 2003; Fall et al., 1998).

Hereditary effects on resistance to fractures decrease with age as environmental factors become the major predictors of increased fractures between the sixth and eight decade of life (Michaelsson et al., 2005; Ralston and de Crombrugghe, 2006). The age differences between our control and GID groups therefore constitute a potential complication, although we have carefully adjusted for age-related differences.

In conclusion, our EO-GID patients displayed a “cross-sex” pattern in the BMD and TBF parameters at baseline, with a “feminized” pattern of male patients and a “masculinized” pattern of female patients. The sex differences between male and female patients were less pronounced than between male and female controls. To our knowledge, this is a novel finding that differs from those of recent publications (Dittrich et al., 2005; Ruetsche et al., 2005). The observed “cross-sex” pattern may be due to a genetic predisposition that may have become visible by our inclusion criteria (Coolidge et al., 2002) or due to an interaction with environmental factors. Our results indicate that, along with the dramatic changes of hormonal blood parameters and their known metabolic implications, M to F GID patients may be at risk of developing osteoporosis and should be monitored accordingly (Gooren, 2005; Michel et al., 2001).

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